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NEUTRALIZATION AND BIODEGRADATION OF SULFUR MUSTARD

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February 1997

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DEPARTMENT OF THE ARMY
U.S. Army Edgewood Research, Development and Engineering Center
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The chemical warfare agent sulfur mustard was hydrolyzed to products that were biologically mineralized in sequencing batch reactors seeded with activated sludge. Greater than 90% carbon removal was achieved using laboratory scale bioreactors processing hydrolyzed munitions-grade sulfur mustard obtained directly from the U.S. Chemical Stockpile. The bioreactor effluent was nontoxic and contained no detectable sulfur mustard or priority pollutants. The sulfur mustard hydrolysis/biodegradation process has potential application to the congressionally mandated disposal of sulfur mustard stockpiles.

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PREFACE

The work described in this report was authorized under the Demilitarization Alternative Technologies Program funded by the Program Manager for Chemical Demilitarization. This work was started in October 1995 and completed in June 1996.

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NEUTRALIZATION AND BIODEGRADATION OF SULFUR MUSTARD

1. INTRODUCTION

Sulfur mustard ("mustard gas", 2,2'-dichlorodiethylsulfide, Chemical Abstract Services number 505-60-2, military designation: HD) was first produced and weaponi ed by the German Army in World War I and has since been stockpiled by several countries including the United States. HD is an oily liquid at room temperature (boiling point 217° C, free ing point 14.45° C). It is a powerful vesicant which affects the eyes and lungs, blisters the skin and is considered a carcinogen (Ward, 1975). HD also exhibits cytotoxic action on hematopoietic tissue, and, at high doses, can be lethal (human oral LD_{50} of 0.7 mg/kg according to the U.S. Army material safety data sheet). HD has previously been used as a chemical warfare agent (Black et al., 1993 and 1994).

The disposal of the existing HD stockpiles presents several technical and political challenges. In 1985, the U.S. Congress directed the Department of Defense to destroy at least 90 percent of the unitary chemical agent stockpile which includes HD (Public Law 99-145). This program was subsequently expanded to include the entire U.S. unitary chemical stockpile. In 1988, the Army, as documented in its Final Programmatic Environmental Impact Statement (U.S. Department of the Army, 1988), decided against the transportation of the existing untreated stockpiles to one or more central facilities and recommended destruction of the stockpiles to be accomplished at each stockpile site. By way of the National Defense Authori ation Act for Fiscal Year 1993, the deadline for completion was set at December 31, 2004.

In 1982, the U.S. Army selected incineration as the preferred technology for destruction of the chemical stockpile. However, the public has frequently expressed reservations about the use of incineration for chemical weapons disposal. As a result, under Public Law 102-484, the Army was directed by Congress to report on Alternative Technologies for the disposal of chemical weapons stockpiles. Neutrali ation followed by biodegradation was one approach which the National Research Council recommended for further research and development (National Research Council, 1994).

The advantages of using hydrolysis as a neutrali ation reaction preceding biodegradation of HD include aqueous medium, complete dechlorination and products (primarily alcohols) which are biodegradable. Also, the hydrolysis reaction does not add

any additional carbon that would require subsequent biological removal. Hydrolysis has been previously utili ed for the detoxification of Canadian HD stockpiles (Reichert, 1975).

Biodegradation has widespread application in municipal wastewater treatment plants, is conducted at ambient temperatures and offers a favorable mass balance for the process.

2. MATERIALS AND METHODS

2.1. Chemical Agent Standard Analytical Reference Material HD

Chemical Agent Standard Analytical Reference Material (CASARM) HD (lot # HD-U-2325-CTF-N) was used as received and was certified as 97.5 +/- 0.2% pure.

2.2. [^{13}C] HD

[^{13}C] HD was custom synthesi ed by the Illinois Institute of Technology Research Institute. ^{1}H and ^{13}C NMR analysis showed it to be 99.8% pure chemically. The isotopic purity was 98.7% on the alpha carbon and 98.5% on the beta carbon.

2.3. Ton Container HD

For larger scale reactions, HD was obtained directly from a one-ton storage container from the U.S. Army Aberdeen Proving Ground chemical stockpile. It was determined to be 89.2 area % pure by gas chromatography/mass spectrometry (GC/MS) and 96.5 area % pure by NMR. The major impurity (4.7%) was (1,2-bis [2-chloroethylthioethane]), also known as compound Q or sesquimustard. The second most predominant impurity was dichloroethane (2.4%) which is probably formed as a thermal decomposition product from the HD dimer. The next most predominant products (2.0%) were the combined isomers of $ClCH_2CH_2SCH_2CH_2CH_2CH_2Cl$ which are believed to be thermal decomposition products of the H-2TG and CH-TG sulfonium ions by way of reaction with chloride ion (Rohrbaugh et al., 1989). The HD was used as received for all hydrolysis and biodegradation studies.

2.4. Modified Wolin Salts Solution

Modified Wolin Salts Solution was prepared 100x as follows: 3.0 g/L nitrilotriacetic acid, 6.0 g/L $MgSO_4.7H_2O$, 0.5 g/L $MnSO_4$,

0.5 g/L $FeSO_4.7H_2O$, 0.1 g/L $CaCl_2.6H_2O$, 0.1 g/L $CoCl_2.6H_2O$, 0.1 g/L $ZnSO_4.7H_2O$, 0.02 g/L H_3BO_3, 0.01 g/L $CuSO_4.5H_2O$ and 0.01 g/L $MoO_4.2H_2O$.

2.5. HPLC Analysis

Thermomixer experiments, NMR experiments and thiodiglycol (TDG) High Pressure Liquid Chromatography (HPLC) analyses were all performed as described elsewhere (Beaudry et al., 1994).

2.6. Gas Chromatography/Flame Photometric Detector Analysis

Gas chromatography/flame photometric detector (GC/FPD) analysis of HD was conducted using a Hewlett-Packard Model 5890 GC with FPD detector and a sulfur filter. Quantitation was via HP3365 Chemstation software. A HP 5 capillary column (10 meters x 0.530 mm i.d. x 2.65 µm film thickness) was used with helium carrier gas at 2.20 mL/min constant flow mode (split ratio 45:5). Injection port temperature was 200° C, oven temperature was 180° C isothermal and the detector temperature was 200° C. $CHCl_3$ or $CDCl_3$ were used as solvents. A linear regression plot of the square root of the area of the 1.8 minute peak gave a correlation coefficient of 1.0000 at HD concentrations of 0.156 micromolar to 5 millimolar. Samples with concentrations greater than 5 millimolar were diluted in $CHCl_3$.

2.7. Nuclear Magnetic Resonance Spectroscopy Analyses

NMR analyses were conducted using a Varian VXR-400S Fourier Transform (FT) NMR spectrometer which operates at 400 MHZ for ^1H observation and at 100 MHZ for ^{13}C observation. All spectra were obtained at probe temperature (22 +/- 1° C) with double precision data accumulation. Samples were provided in $CDCl_3$, D_2O or water. All samples run in $CDCl_3$ were referenced to internal tetramethylsilane standards using the $CHCl_3$ resonance (δ^1H, 7.24; $\delta^{13}C$, 77.2) as a secondary reference. ^1H spectra in D_2O and water were referenced to external sodium 3-trimethylsilylpropionate-2,2,3,4-d_4 (TSP) in D_2O. Quantitative data were obtained by digital integration of peak areas.

Survey ^1H spectra were run for product identification using a sweep width of 8000 H (20 ppm), a pulse width of at least 12 microseconds (30 degrees), an acquisition time of 2-4 seconds and a pulse delay of 2-4 seconds. Corresponding ^{13}C spectra were acquired using a sweep width of at least 25000 H (250 ppm), an acquisition time of 1.6 seconds, and a pulse delay of 3 seconds.

Spectra were accumulated until the desired signal-to-noise ratio was achieved.

Spectra obtained for kinetic runs were acquired with fixed gain and integral values using the absolute intensity (AI) mode of the spectrometer. Each set of samples from a reaction were analy ed under identical operating conditions so that integral area from different samples from the set could be directly compared. For ^{1}H spectra, 16 transients were collected using a sweep width of 8000 H (20 ppm), an acquisition time of 2.0 seconds, a pulse width of 7 microseconds (21 degrees) and a pulse delay of 2 seconds. For ^{13}C spectra, 64 transients were collected using a sweep width of 25000 H , an acquisition time of 2 seconds, a pulse width of 12 microseconds (90 degrees), and a pulse delay of 2.5 seconds. ^{1}H data obtained in $CDCl_3$ were normali ed to the residual $CHCl_3$ resonance in the solvent. Data obtained in water or D_2O were not normali ed.

3. RESULTS AND DISCUSSION

3.1 Hydrolysis in NaOH Solutions

HD is highly insoluble in aqueous solutions (Yang et al., 1988) and toxic to microorganisms due to its reactivity with en ymes and other proteins (Bush, 1946, pp. 431-439 for review). For these reasons HD is a poor candidate for direct biodegradation. However, HD does react with water to form hydrolysis products which are largely soluble in water and are therefore far better candidates for biodegradation.

The mechanism of mustard hydrolysis at ambient temperature has been investigated previously (Bartlett and Swain, 1949, Helfrich and Reid, 1920, McManus et al., 1985, Yang et al., 1987a, Yang et al., 1988, Yang et al., 1987b.). It has been demonstrated that HD reacts through a series of sulfonium ion intermediates to produce thiodiglycol. However, additional ether or thioether products can also form depending on the actual conditions of the reaction. In dilute HD solutions, with low chloride ion concentrations and in the presence of a polar organic solvent (i.e. 5 vol % acetone), the two step hydrolysis rate constants (Bartlett and Swain, 1949) are $k_1 = 2.35 \times 10^{-3}\ s^{-1}$ and $k_2 = 4.33 \times 10^{-3}\ s^{-1}$ at 25° C. However, the rate of hydrolysis in pure water is limited by the rate of mass transfer because of the insolubility of HD in aqueous solution. Consequently, dissolution and reaction take place simultaneously (Yang et al., 1988). However, in a previously-studied two phase system, dissolved and

unreacted HD could not be detected in pure water (Yang et al., 1988). HD is hydroly ed at the interface, and the hydrolysis products then dissolve in water. Therefore, agitation during hydrolysis is a critical factor and any measurements of the hydrolysis rate must account for the degree of agitation. Both the rate of mass transfer and the rate of hydrolysis can be accelerated at elevated temperatures.

3.2. Effect of Temperature on the Rate of HD Hydrolysis

Because HD dissolution and hydrolysis are essentially simultaneous, absolute hydrolysis rates cannot be measured in this aqueous system containing no other solvent. The observed rate, expressed as HD disappearance over time, is a function of both the rate of dissolution and the rate of hydrolysis. However, when agitation is controlled and temperature is varied, the relative observed hydrolysis rates can be obtained by monitoring the disappearance of HD over time.

In the presence of NaOH, HD hydrolysis actually encompasses two separate reactions: hydrolysis (presented in its simplest form in Equation 1) and neutrali ation of the HCl produced in the hydrolysis step (Equation 2). The overall hydrolysis and neutrali ation reaction is illustrated in equation 3.

1. $ClCH_2CH_2SCH_2CH_2Cl + \underline{2}H_2O \text{ ------> } HOCH_2CH_2SCH_2CH_2OH + \underline{2}HCl$

2. $\underline{2}HCl + \underline{2}NaOH \text{ ------> } \underline{2}H_2O + \underline{2}NaCl$

3. $ClCH_2CH_2SCH_2CH_2Cl + \underline{2}H_2O + \underline{2}NaOH \text{ ------> } HOCH_2CH_2SCH_2CH_2OH + \underline{2}NaCl + \underline{2}H_2O$

CASARM HD (1.27 mg/ml) was hydroly ed in 0.5 mL of a 20 mM solution of aqueous NaOH for varying lengths of time at 30, 40, 50, 60, 70, 80 and 90° C. Agitation and temperature were controlled by conducting all reactions in separate tubes in a Thermomixer (a heating block with agitation control). Each reaction was quenched and extracted in its entirety by the addition of an equal volume of $CHCl_3$. This approach precluded the need to withdraw aliquots from the two-phase reaction system. The $CHCl_3$ extracts of each entire reaction were analy ed by GC/FPD. The slopes of the lines representing the square roots of the peak areas versus time were plotted versus temperature (Figure 1.a.) then as an Arrhenius plot (Figure 1.b.). The hydrolysis rate at 70° was more than 28 times the rate at 30° C; rates at 80° and 90°

were too fast to measure by this method. The enthalpy of activation for the HD hydrolysis reaction was calculated as 17.9 Kcal/mol. This is similar to other HD enthalpy of activation values previously reported using other techniques (Brookfield et al., 1943; Hopkins, 1919; Mohler and Hartnagel, 1941; Peters and Walker, 1923; Yang et al., 1987).

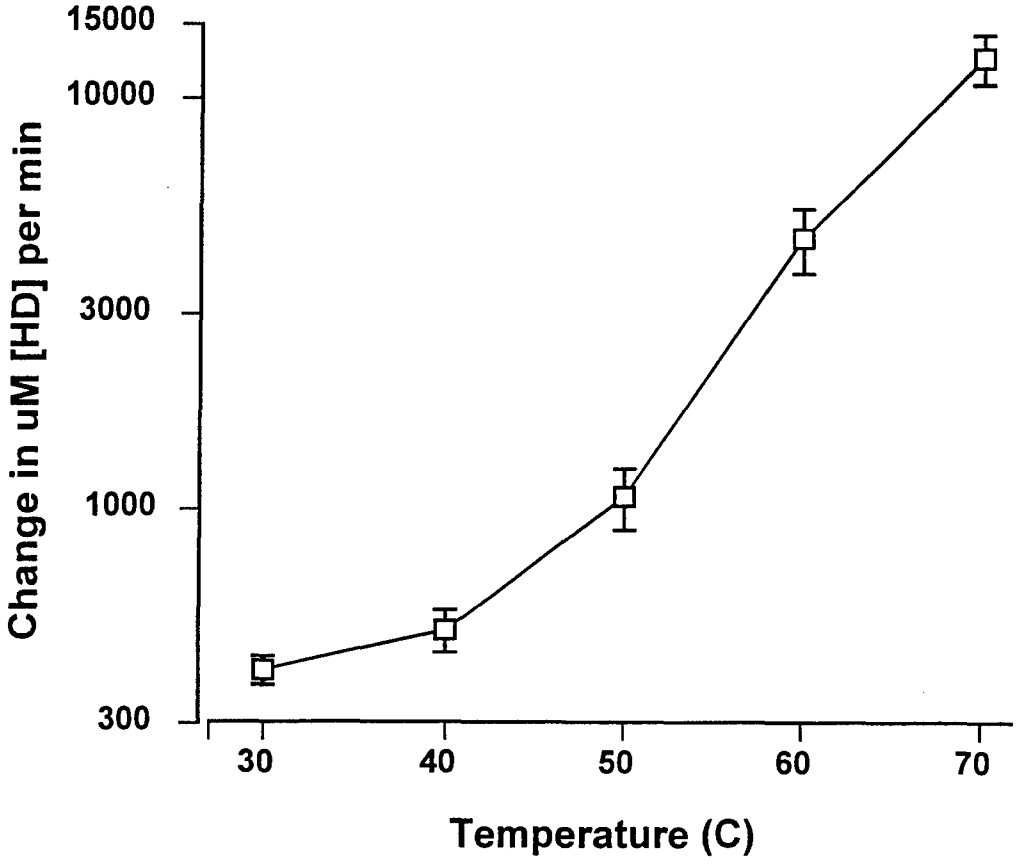

Figure 1.a. HD hydrolysis rate vs. temperature.

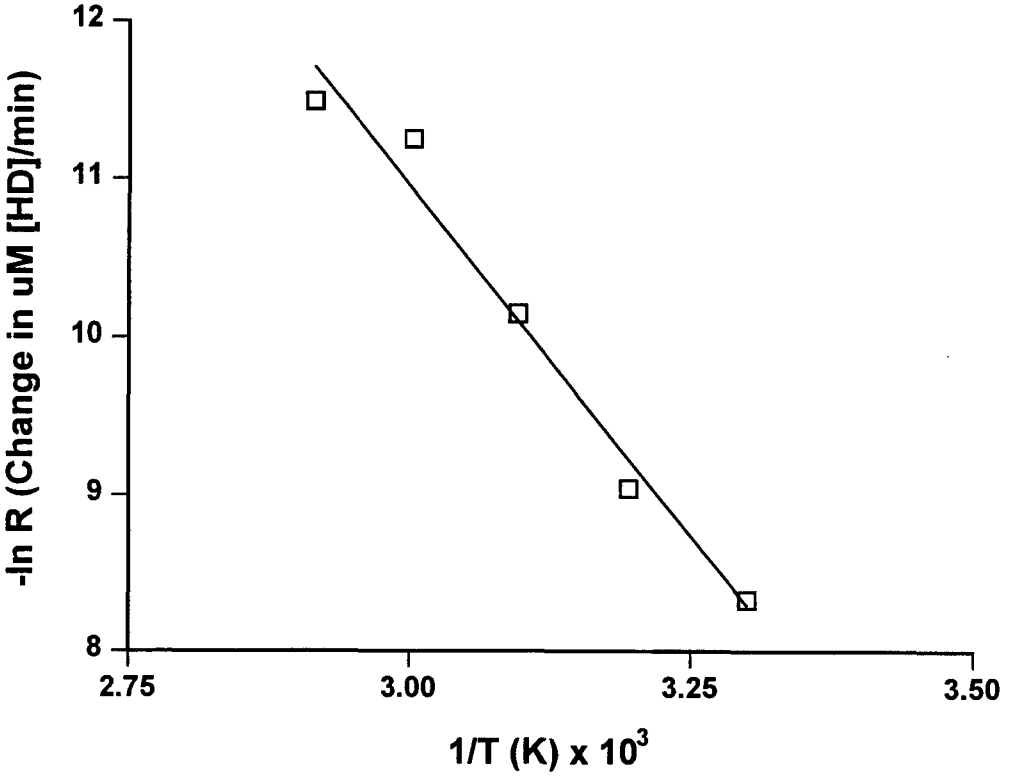

Figure 1.b. Arrhenius plot of observed HD hydrolysis rates at 30, 40, 50, 60 and 70° C.

In practice, caustic hydrolysis yields a distribution of ether-alcohol, thioether-alcohol and crown ether compounds in addition to TDG (Beaudry et al., 1994). Most of these compounds are of the generali ed structures $HO-CH_2-CH_2-S-(CH_2-CH_2-S)_x-CH_2-CH_2-OH$ or $HO-CH_2-CH_2-S-(CH_2-CH_2-O)_x-CH_2-CH_2-OH$ where x is typically equal to 1 or 2.

The product distribution is affected by temperature. Specifically, the yield of TDG, relative to ether-type compounds, increased at higher reaction temperatures. Reactions were conducted at different temperatures by adding HD through a separatory funnel over a period of 30 minutes to a solution of aqueous NaOH containing 2.1 moles of NaOH for each mole of HD at different temperatures ranging from 90 to 30° C. Total reaction time was two hours at 90°, three hours at 70°, four hours at 50° and five hours at 30°. Lower temperatures of reaction yielded larger fractions of ethers and smaller fractions of TDG (Figure 2). For purposes of analysis, the peak areas of all ether-type compounds were integrated together to permit a direct comparison with the TDG concentration.

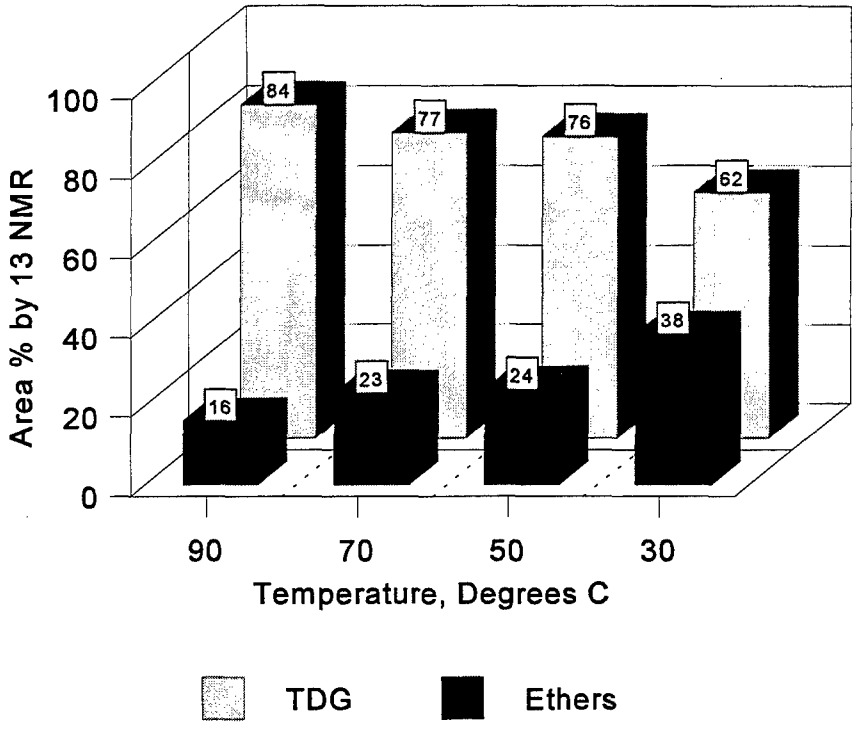

Figure 2. HD hydrolysis products as a function of temperature of the reaction.

The starting concentration of HD in the reaction also affects the product yield. Reactions were conducted with different HD starting concentrations at 90° C in a roundbottom flask for two hours. HD addition was over a period of 30 minutes from a separatory funnel and agitation was via Teflon paddle and an overhead stirrer. Product analysis by NMR showed that the starting HD concentration was inversely proportional to the final TDG yield over a range of concentrations between at least 1% and 20% (Figure 3).

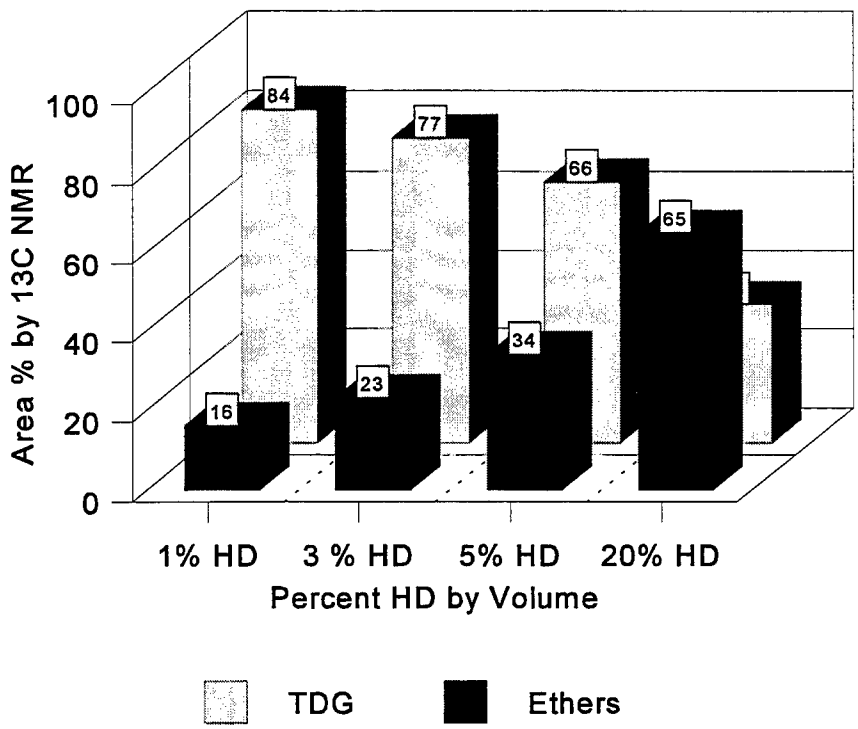

Figure 3. HD hydrolysis products as a function of HD concentration in the reaction. All reactions were conducted with 2.1 moles of NaOH per mole of HD at 90 degrees C for 2 hours.

3.3. Hydrolysis in Water

HD can also be hydroly ed in hot water with no base present. Two equivalents of HCl are produced which reduce the pH to very low levels. The product is then neutrali ed with NaOH upon cooling. This reaction produces a higher yield of TDG and a lower yield of ethers than the caustic reaction (Figure 4). This is potentially advantageous in that TDG is non-toxic and readily biodegradable. It is also miscible in water which ensures a single phase product.

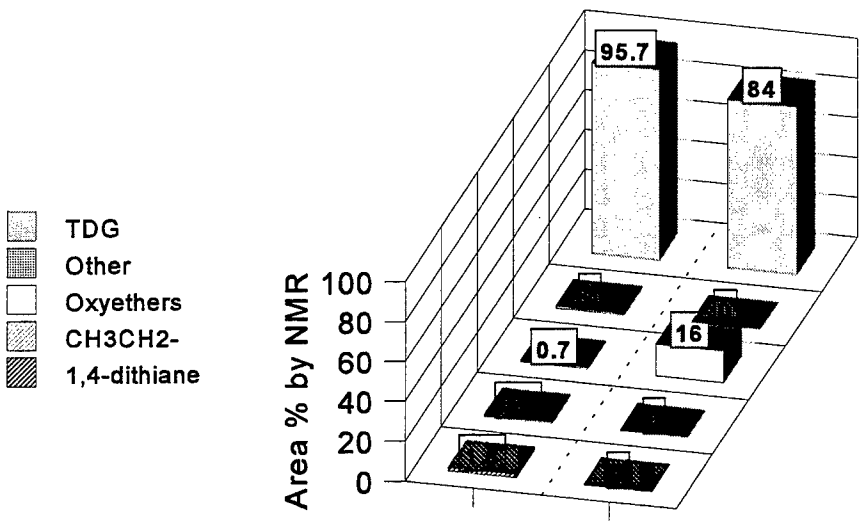

Figure 4. NMR Analyses of the Products of HD Hydrolysis in Water (Left Row) and Aqueous NaOH (Right Row)

As mentioned above, HD hydrolysis proceeds through a series of sulfonium ion intermediates. The relative amounts of two of these sulfonium ions (H2-TG and CH-TG, Figures 5.a and 5.b, respectively) and TDG vs. time of reaction are illustrated in Figure 5.c for the case of the reaction of 1% HD with water at 100° C. This experiment was conducted in a 3 L roundbottom flask with agitation via Teflon paddle with a top-mounted motor

operating at 200-300 rpm. HD (30 ml) was added through the top of the flask in a single batch. When all the HD had disappeared visually (approximately 5 minutes after addition), samples were removed from the reaction mixture and immediately cooled in an ice bath. Samples were stored on ice until NMR analysis for TDG and sulfonium ions. Results (Figure 6.) indicate that TDG was the predominant product (84.8%) present in the first sample taken immediately after the HD disappearance was complete. The residual sulfonium ions continued to hydroly e to produce a final TDG yield of 92.5% after an additional 15 minutes of reaction. In the course of the hydrolysis reaction, H2-TG hydroly es to form CH-TG and TDG.

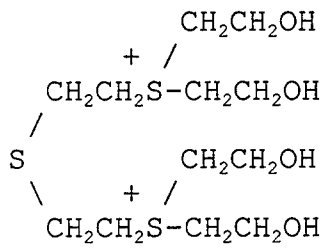

Figure 5.a. Structure of H2-TG sulfonium ion

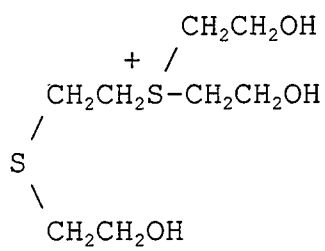

Figure 5.b. Structure of CH-TG sulfonium ion.

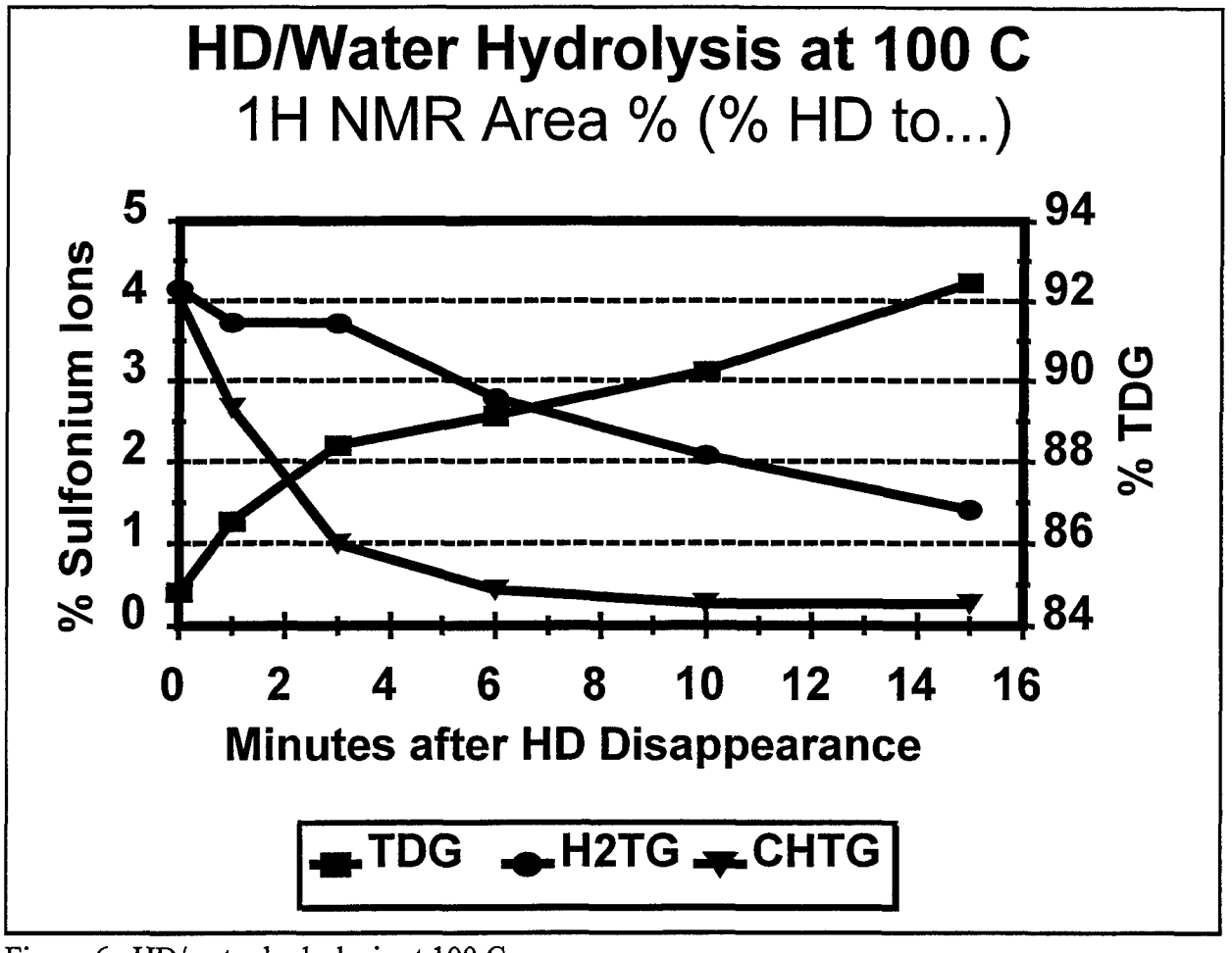

Figure 6. HD/water hydrolysis at 100 C.

3.4. Biodegradation

In order to meet international treaty requirements (Report of the conference on disarmament to the General Assembly of the United Nations, 1992), it is necessary to not only detoxify chemical agents but also to destroy them irreversibly. In order to address this issue of final disposal, biodegradation of the hydroly ed HD products was studied in a sequencing batch reactor (SBR). SBRs offer several important operational advantages for the treatment of chemical wastes (Irvine and Ketchum, 1989 for review). First, they are efficient. The SBR models as a continuous flow stirred tank reactor (CFSTR) followed by a plug flow reactor, offering the ideal volumetric reactor configuration for unsteady state activated sludge systems. Second, they operate in the batch mode which not only offers a kinetic advantage over conventional CFSTRs (therefore smaller si e) but, for ha ardous waste treatment, they permit batch analysis and toxicity testing prior to discharge. Third, they use a single tank for both treatment and settling; therefore, no secondary

clarifier is required. Finally, they are robust and flexible. SBRs are intentionally operated over a range of substrate concentrations, pH conditions and oxygen concentrations, thereby allowing selection of a very diverse and robust population of microorganisms. Operational strategies can also be varied to accomplish carbon, nitrogen and/or phosphorus removal.

3.4.1. Feedstock Preparation (HD Hydrolysis)

HD for all biodegradation studies was obtained directly from the U.S. Chemical Stockpile at Aberdeen Proving Ground, MD and was used exactly as received from the one ton storage container. It was routinely hydroly ed for two hours at 90° C at a concentration of 1% (vol/vol) in either 0.67% NaOH (wt/vol) or tap water. A 3 L roundbottom, water-jacketed flask agitated with a motor-driven Teflon paddle was used for the reactions. HD was added over a period of 30 minutes from a separatory funnel at the top of the flask. In addition to the expected organic products a solid precipitate was formed. Electron microscopy analysis indicated the material was comprised primarily of iron complexes. The iron was presumably derived from the steel container in which the HD had been stored for approximately 40 years. The mass of the precipitated material was equivalent to less than 1% of the mass of the starting HD. The HD/water hydrolysis reaction also produced a slightly smaller quantity of a similar material after pH neutrali ation with NaOH. Hydrolysate solutions were routinely analy ed for HD by extracting 50 ml of hydrolysate with 1 ml $CHCl_3$ followed by GC/MS analysis of the extract to a detection limit of 160 parts per billion (ppb). The U.S. Army drinking water standard for HD is 200 ppb. All solutions which were hydroly ed and analy ed by these techniques were found to contain no detectable HD at the 160 ppb level. Solutions were also routinely analy ed by NMR to determine the relative area percent of TDG and ethers and also for sulfonium ions.

3.4.2. Sequencing Batch Reactor Operation

Bioreactors were operated as sequencing batch reactors (SBRs) on 24 hour cycles. Seed cultures were obtained from activated sludge (Back River Wastewater Treatment Plant, Baltimore, MD). Initial mixed liquor suspended solids (MLSS) levels were approximately 2800 ppm. Modified Wolin Salts solution (10 ml/L) was added to provide inorganic micronutrients along with 1445 mg/L NH_4Cl and 278 mg/L KH_2PO_4 as nitrogen, potassium and phosphate sources. Hydroly ed HD was provided as the sole

source of carbon and sulfur. Operating pH was maintained between 6.5 and 8.5, primarily by the addition of 15 g/L of $NaHCO_3$ as a buffer. Also, NaOH was occasionally added on demand through a pH controller when the pH dropped below 6.5. Minerali ation of sulfur-containing compounds such as TDG causes acid production when the sulfur is converted to sulfate which must be charge-balanced with two cations.

A six liter SBR was operated with the caustic-hydroly ed HD as feed for two months. Figure 4 includes a typical NMR analysis of the feedstock used for the operation of these bioreactors.

The reactor was normally operated as follows: 4 hour aerated FILL, 18 hour aerated REACT, 1.5 hour SETTLE and 0.5 hour DRAW. Hydraulic residence time (HRT) was 16 days, feed total organic carbon (TOC) level was 3400 to 3800 mg/L and MLSS increased from the initial level of 2.8 g/L to 3.5 g/L. Effluent was analy ed periodically (~3-5 times per week) for TOC, TDG concentration by High Performance Liquid Chromatography (HPLC) and two or three times a week for effluent suspended solids (ESS). Although TDG was typically removed to levels >99% (as determined by HPLC analysis), the effluent TOC continued to rise throughout three HRTs, eventually reaching levels of greater than 1000 ppm, corresponding to ~70% carbon removal efficiency. Because of the high effluent TOC, this reactor was discontinued. in favor of a reactor which was fed the water (acid) hydroly ed feed which contained a greater percentage of TDG and less ether-type compounds.

A second, 12 liter SBR was started, seeded with the same activated sludge and fed with water (acid) hydroly ed feed. This SBR was started and operated under the same conditions as those used for the caustic-hydroly ed feed SBR. The reactor with water-hydroly ed feed was continuously operated for a total of 146 days. After 100 days the reactor si e was reduced from 12 L to 5 L and the FILL and DRAW volumes were reduced proportionally. All other aspects of the operation of the reactor were maintained. Figure 4 illustrates a typical NMR analysis of the water hydroly ed feed used for this bioreactor. It differs from the caustic hydroly ed feed primarily in the ratio of TDG to ethers; the water hydroly ed material contained more TDG and fewer ether products. A 146 day average of operational data from this bioreactor is presented in Table 1. Data include the values from occasional upset conditions due to operator error or equipment malfunction. Average effluent suspended solids, effluent TDG and effluent TOC would all be somewhat lower if

upset conditions were not included in the average.

Test	Value	# Measurements
Feed TOC	3749 ppm	1 per batch
MLSS	4163 ppm	66
Sludge Volume Index	94.4	2
Endogenous OUR*	14.5 mg/L/hr	19
Endogenous SOUR*	4.2 mg/g/hr	15
OUR after feed spike	32.6 mg/L/hr	17
Effluent TOC	345 ppm	82
Effluent TDG (vol/vol)	0.003%	85
Effluent Suspended Solids	207 ppm	65
Average Hydraulic Residence Time	12.93 days	3
Efficiency of TDG Removal	99.6%	12
Overall Efficiency of Carbon Removal	90.6%	13

Table 1. Average operational values over 146 day period for HD/water bioreactor.
*oxygen uptake rate
**specific oxygen uptake rate

MICROTOX analysis of the effluent showed it to be non-toxic (100% effluent produced no changes in the luminescence levels of the test bacteria). Toxicity characteristic leachate protocol (TCLP) analysis of the effluent found none of the tested organics (Table 2):

TCLP Organics Tested None Detected at the Detection Limits in mg/L in Parentheses	
pyridine (0.1)	tetrachloroethylene (0.1)
lindane (0.07)	toxaphene (0.12)
carbon tetrachloride (0.1)	trichloroethylene (0.1)
chlordane (0.007)	2,4,6-trichlorophenol (0.1)
chloroben ene (0.1)	vinyl chloride (0.2)
chloroform (0.1)	2,4-D (1.2)
1,4-dichloroben ene (0.1)	methoxychlor (0.088)
1,2-dichloroethane (0.1)	ben ene (0.1)
1,1-dichloroethane (0.1)	MEK (2.0)
2,4-dinitrotoluene (0.1)	hexachloroben ene (0.1)
hexachlorobutadiene (0.1)	total cresols (0.1)
hexachloroethane (0.1)	heptachlor epoxide (0.0042)
nitroben ene (0.1)	heptachlor (0.0015)
2,4,5-trichlorophenol (0.1)	endrin (0.003)
pentachlorophenol (0.5)	2,4,5-TP (0.17)

Table 2. TCLP organics tested. None were detected.

Two of these compounds, vinyl chloride and 1,2-dichloroethane, were of particular interest since 1,2-dichloroethane is found as an impurity in the HD used for these experiments (Beaudry et al., 1994) and vinyl chloride is a hydrolysis product of 1,2-dichloroethane.

The results of the TCLP analysis for metals are presented in Table 3:

TCLP Analysis for Metals		
Metal	**Amount (ppm)**	**Detection Limit**
silver	None	0.01
arsenic	None	0.1
barium	0.11	0.01
cadmium	0.21	0.01
chromium	None	0.02
lead	None	0.1
selenium	None	0.07
mercury	None	0.01

Table 3. Results of TCLP analysis for metals.

3.5. Material Balance Calculations

With some simplifying assumptions, a mass balance can be calculated for the HD hydrolysis/biodegradation process (Figure 7). These assumptions and their explanations are:

1. 100% pure HD. In reality, the sample which was obtained from the one-ton storage container from the U.S. Chemical Stockpile was 89% pure by GC/MS. The most abundant impurities had empirical formulas similar to HD (MATERIALS AND METHODS) and would therefore have little overall effect on the mass balance.

2. 100% conversion of HD to thiodiglycol. 89% pure HD which was hydroly ed under acidic conditions in hot water produced thiodiglycol at a yield of approximately 95%, indicating that some of the HD impurities were also converted to TDG. Also, the empirical formula of the other products was generally similar to that of thiodiglycol.

3. 50/50 conversion of carbon to CO_2 and biomass. This value is empirical and approximate, based on the results of other similar biological systems.

4. A biomass empirical formula of $C_5H_{9.615}O_{1.5024}N_{1.202}P_{0.1164}S_{0.0375}$. This value is based on that measured for *Escherichia coli*

(Ingraham et al., 1983).

1. Hydrolysis:

$ClCH_2CH_2SCH_2CH_2Cl + \underline{2}\ H_2O \rightarrow HOCH_2CH_2SCH_2CH_2OH + \underline{2}\ HCl$

HD **TDG**

2. Neutralization:

$\underline{2}\ HCl + \underline{2}\ NaOH \rightarrow \underline{2}\ NaCl + \underline{2}\ H_2O$

Water consumed in first step is regenerated here.

3. Biodegradation:

$HOCH_2CH_2SCH_2CH_2OH + \underline{4.613}\ O_2 + \underline{0.047}\ KH_2PO_4 + \underline{0.481}\ NH_4Cl + \underline{2.429}\ NaOH$

TDG **K, P Source** **N Source**

$\rightarrow \underline{0.4}\ C_5H_{9.615}O_{1.5024}N_{1.202}P_{0.1164}S_{0.0375} + \underline{2.459}\ NaCl + \underline{5.3}\ H_2O + \underline{2}\ CO_2 + \underline{0.985}\ Na_2SO_4$

Biomass

$+ \underline{0.022}\ KCl$ (This includes the 2 moles of NaCl generated during step 2).

Figure 7. Mass balance calculations for HD hydrolysis/biodegradation process.

Calculated per ton of HD influent, the total influent and effluent would be as shown in Table 4.

Tons Input (3.24 total)			Tons Output (3.24 total)	
HD	1.00		CO_2	0.55
NaOH	1.11		H_2O	0.60
O_2 (from air)	0.93		Biomass	0.30
NH_4Cl	0.16		Na_2SO_4	0.88
KH_2PO_4	0.04		NaCl	0.90
			KCl	0.01

Table 4. Total input and output for process.

* This figure represents only the water actually produced from the oxidation reactions, not the larger volume of carrier water in which the reactions occur.
** Dry weight of biomass. This value would presumably be reduced 30-40% by digestion.

4. CONCLUSIONS

HD hydrolysis may be used to destroy HD under either caustic or acidic conditions. When NaOH is added to the reaction, a mixture of thiodiglycol and ether-alcohol type compounds is produced. The relative amounts of thiodiglycol produced are greater at higher temperature and under conditions of greater aqueous dilution. The hydrolysis reaction was complete; HD was not detected with a detection limit of 160 ppb.

HD hydrolysis products can be biodegraded using activated sludge in a sequencing batch reactor. The efficiency of carbon removal was greater when products from the acidic vs. the caustic reaction were used as feed.

Blank

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